Regular exposure to rabies virus and lack of symptomatic disease in Serengeti spotted hyenas

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We report a previously unrecognized complexity to the ecology of rabies in wildlife. Rabies-specific virus-neutralizing antibodies in spotted hyenas, the most numerous large carnivore in the Serengeti ecosystem (Tanzania, East Africa), revealed a high frequency of exposure of 37.0% to rabies virus, and reverse transcriptase (RT) PCR demonstrated rabies RNA in 13.0% of hyenas. Despite this high frequency, exposure neither caused symptomatic rabies nor decreased survival among members of hyena social groups monitored for 9 to13 years. Repeated, intermittent presence of virus in saliva of 45.5% of seropositive hyenas indicated a ''carrier'' state. Rabies isolates from Serengeti hyenas differed significantly (8.5% sequence divergence) from those isolated from other Serengeti carnivores, suggesting that at least two separate strains circulate within the Serengeti carnivore community. This finding is consistent with the fact that exposure in hyenas increased with age and social status, following a pattern predicted by intraspecific age and social-status-dependent oral and bite contact rates. High seroprevalence of rabies, low basic reproductive rate of the virus (R_0) of 1.9, a carrier state, and the absence of symptomatic rabies in a carnivore in an ecosystem with multihost and multistrain maintenance has not been previously demonstrated for rabies. Because of the substantial differences between the hyena viral isolates and those from canids and viverrids in the Serengeti, it is unlikely that spotted hyenas were the source of rabies virus that killed several African wild dog packs in the Serengeti ecosystem in the 1990s.

The ecology of rabies in wildlife populations and natural ecosystems is poorly understood (1, 2). In Africa, where canid rabies predominates (3), clinical cases of rabies are apparently rare or absent in national parks, even when present in surrounding areas (4, 5). If this impression is correct, it has important implications for the assessment of the threat of rabies to wildlife in major African biodiversity sites. We therefore ask the question, is the virus not present in wildlife populations in national parks, are clinical cases not recognized, or is the virus maintained in coevolved, enzootic host–parasite relationships (2, 6, 7)?

The Serengeti ecosystem is a World Heritage Site, a Biosphere Reserve, and an important area for the conservation of African carnivores (8). Although rabies has been diagnosed in domestic dogs (9) and African wild dogs (*Lycaon pictus*; refs. 9 and 10) in areas surrounding the Serengeti National Park, Tanzania, and bat-eared foxes (*Otocyon megalotis*; ref. 11) within the Park, knowledge of the ecology of rabies in the Serengeti ecosystem is scant. Here we report an unrecognized complexity to the ecology of rabies in a major African ecosystem. We use data from a 13-year study of the most numerous large carnivore in the ecosystem, the spotted hyena (*Crocuta crocuta*; ref. 12), and combine these with disease surveillance data from the carnivore community within the Park.

Materials and Methods

Serengeti Hyenas. Several hundred individually recognized (12–14) Serengeti hyenas in three social groups were regularly monitored in terms of behavior and demography for 13, 10, and 9 years, respectively, between May 1987 and June 2000 during more than 15,000 h of observation. Serengeti hyenas live in large social groups (clans) with a mean number of 45 adults and subadults (12) at a density of 0.8 adults and subadults per km^2 with linear female and male dominance hierarchies (12, 13) in defended territories with female philopatry and male dispersal (12–15). Cubs are reared in a communal den inside the clan territory and nursed by their mother (12) in the vicinity of the den (15). During 46–62% of the year, all clan members other than den-bound cubs regularly travel (commute) on average 40 km from their territory to forage in areas containing large migratory herds (14, 15). Subadults had a lifetime range similar to adult females, who they repeatedly accompanied on commuting trips, whereas reproductively active, immigrant males typically ranged more widely (13, 15).

Age at blood and tissue sampling and longevity (in years) were determined from known life-histories of individually recognized study animals (12, 13). Spotted hyena social status was scored as standardized rank (−1 for lowest ranking, +1 for highest-ranking individual) using the outcome of dyadic interactions (13). The rate at which individuals had their open mouths licked by other clan members (oral contact rate) were calculated for 32 cubs, 9 subadults, 12 immigrant males, and 26 adult females that were observed by focal sample (mean ± SEM, 73 ± 5 min) for more than 100 h at the communal den. Rates at which 112 adult males and 171 adult females received bites from conspecifics that broke the skin (bite contact rate) were calculated from observations of wounds received between 1991 and 2000.

Tests for Rabies Exposure and Infection. Serum was collected from 59 female, 37 male, and 4 unsexed hyenas from 1988 to 1999 and stored and transported at ≤−10°C. In terms of age classes, sample sizes for seroprevalence were 20 cubs, 13 subadults, and 67 adults. Rabies-specific virus-neutralizing antibody (VNA) titers were determined by the Rapid Fluorescent Focus Inhibition test (RFFIT) (16) using Challenge Virus Standard virus and including the World Health Organization international reference serum to determine international units (IU)/ml. Titers ≥0.5 IU/ml were considered positive (17, 18), and titers exceeding 1.5 IU/ml were considered high. Saliva samples (n = 303) were collected as serial samples from 52 individuals that chewed

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Abbreviations: IU, international units; NCC, neuroblastoma cell culture; RT, reverse transcriptase; VNA, virus-neutralizing antibody; N-P, nucleoprotein–phosphoprotein.

Data deposition: The sequences reported for rabies virus isolates from Serengeti carnivores discussed in this paper have been deposited in the GenBank database (accession nos. AY034155–AY034174).

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Table 1. Proportion of brain samples from which rabies virus was isolated

Species	Family	Positive for rabies RNA by RT-PCR, %	*n*	Murine NCC-positive, %	*n*
Spotted hyena (*Crocuta crocuta*)	Hyaenidae	13.0	23	0.0	57
Bat-eared fox (*Otocyon megalotis*)	Canidae	46.7	15	25.0	16
Black-backed jackal (*Canis mesomelas*)	Canidae	0.0	6	0.0	6
White-tailed mongoose (*Ichneumia albicauda*)	Viverridae	66.7	3	66.7	3
Banded mongoose (*Mungos mungo*)	Viverridae	0.0	3	0.0	3
Slender mongoose (*Herpestes sanguinea*)	Viverridae	0.0	3	0.0	3
Dwarf mongoose (*Helogale parvula*)	Viverridae	0.0	2	0.0	2

Although murine NCC demonstrates the presence of viable virus particles, RT-PCR demonstrates the presence of either viable or nonviable rabies virus particles.

cotton wool swabs (mean ± SEM, 5.1 ± 0.5 samples) and as single samples from a further 37 individuals. Before use, instruments that held swabs were flamed to prevent contamination. Saliva samples were stored and transported in a cold chain of −196°C, and screened by using murine neuroblastoma cell cultures (NCC; ref. 19) and reverse transcriptase (RT)-PCR (20, 21). Brain samples were collected opportunistically from recently dead Serengeti carnivores (n = 57 spotted hyenas, n =33 from other species, Table 1), killed by cars or lions (*Panthera leo*) or that had died from other causes, by inserting a plastic straw into the brain through the occipital foramen. Samples inside straws were stored and transported either in a cold chain of −196°C, or in phosphate-buffered 50% glycerol solution. Brain samples from domestic dogs in areas surrounding the Serengeti and other locations in Tanzania were provided by regional Veterinary Investigation Centers, Tanzania, and the Animal Disease Research Institute, Tanzania, or from domestic dogs culled by rangers in protected areas. In Tanzania, domestic dog samples were stored frozen at either −20°C or in 50% glycerol solution. Brain, tissue, and saliva from all species were checked for virus presence by using NCC and RT-PCR. RFFIT, NCC, and RT-PCR tests were conducted at the Federal Research Centre for Virus Diseases of Animals, Germany.

Nucleotide Sequencing of Viral Isolates. For the RT-PCR, total RNA was isolated from original samples (brain, salivary glands, or saliva) by using the RNeasy Kit (Qiagen, Hilden, Germany). RT and amplification of cDNA by PCR with primers N1161P (5′-AAGAACTTCAAGAATACGAGGC-3′, nucleotides 1161–1182 of the SAD B19 sequence; see ref. 20) and N1579 M (5′-TTCAGCCATCTCAAGATCGG-3′, nucleotides 1579–1560 of the SAD B19 sequence) were performed on 1 μg of total RNA by using the Titan One Tube RT-PCR System (Roche Molecular Biochemicals) following the protocol described in ref. 21. The amplified products were analyzed by ethidium bromide-stained agarose gel electrophoresis, and the resulting bands were excised and purified by using the QIAquick PCR Purification Kit (Qiagen). The purified 400-bp PCR products from the nucleoprotein–phosphoprotein (N-P) gene segment were labeled for sequencing using the ABI Prism Big-Dye Terminator Cycle Sequencing kit (Applied Biosystems), unincorporated fluorescent nucleotides were removed by using the DyeEx Spin kit (Qiagen), and the sequences were collected with an Applied Biosystems Prism 377 automated sequencer. In total, 381 nucleotides of the N-P gene segment for each isolate were sequenced (nucleotides 1199–1579 on the SAD B19 genome). Alignment and basic sequence statistics were done with the GCG package (Sequence Analysis Software Package, version 10.1; Madison, WI). The sequences were compared with published sequences from an African wild dog from north of the ecosystem (10) and an Ethiopian spotted hyena (17), and published (22) and unpublished isolates from database sequence files representing human and animal examples of rabies virus genotype 1 from Europe and the Middle East (database accession numbers starting with AF from Israel and U from elsewhere). Phylogenetic relationships among sequences obtained were estimated by using the neighbor-joining method as implemented in the Phylogeny Inference Package PHYLIP version 3.5 (23).

Basic Reproductive Rate R_0 of the Virus. R_0 of the virus in Serengeti hyenas was calculated for mortality schedule "II" (a newborn individual's probability of surviving to age t declines exponentially with t; ref. 24) by using the adult annual mortality rate μ of Serengeti hyenas of 0.143 ± 0.027 (mean ± SEM; ref. 25) and the age-specific seroprevalence s_a. This schedule is the most appropriate for the survival pattern of Serengeti hyenas, and thus R_0 was estimated from equations 4.26 and 4.32 in ref. 24. In a sensitivity analysis, μ was varied ± SEM and the change in μ was compared with the change in the resulting R_0 value. The result was that R_0 was insensitive to the precise value of μ, because for each 1% change in μ the value of R_0 changed only by 0.40% to 0.58%. Estimates of R_0 calculated with an alternative, less appropriate mortality schedule following equations 4.20 and 4.31 in ref. 24 yielded estimates that were reduced by 20%.

The size of the primary vector population N_v was calculated as $N_v = N \cdot s \cdot s_v/R_0$, with N hyena population size, s overall seroprevalence, and s_v the proportion of seropositive individuals where viral RNA was demonstrated by RT-PCR in saliva samples.

Statistical Analysis. For hyenas blood-sampled twice, one randomly chosen sample was included in the analyses. Unless otherwise stated, all statistical analyses were performed by using SYSTAT 9.0 (26), all degrees of freedom and sample sizes refer to numbers of individuals, and basic statistics are presented as means ± SEM. Calculations (27) of the statistical power β of the survival analysis to detect a significant difference between the survival of seronegative and seropositive individuals were based on the expectation that exposed individuals were less likely to survive than seronegative ones. Expected survival for seropositive animals was based on the (*i*) typical scenario for carnivores such as red foxes (*Vulpes vulpes*; around 8% likely survival; ref. 28) or raccoons (*Procyon lotor*; around 5% likely survival; ref. 1) where most animals die soon after exposure; (*ii*) more benign scenario for Indian mongooses (*Herpestes auropunctatus*) where a substantial minority might survive exposure (around 21% likely survival; ref. 29). Two rabies outbreaks in a population of Kalahari hyenas (30) living at a density (0.008–0.01 individuals per km^2) approximately two orders of magnitude lower than the Serengeti hyenas (14) caused substantial mortality (43% of all causes of death in 12 years of monitoring) and suggest that Kalahari hyenas follow the "red fox scenario." Calculations used an α of 0.05 and the observed n = 26 for each group of seronegative and seropositive animals, and were based on 10,000 simulations (27). For the "mongoose scenario" the simulations yielded β to be 0.96; for the "red fox scenario," β = 0.99.

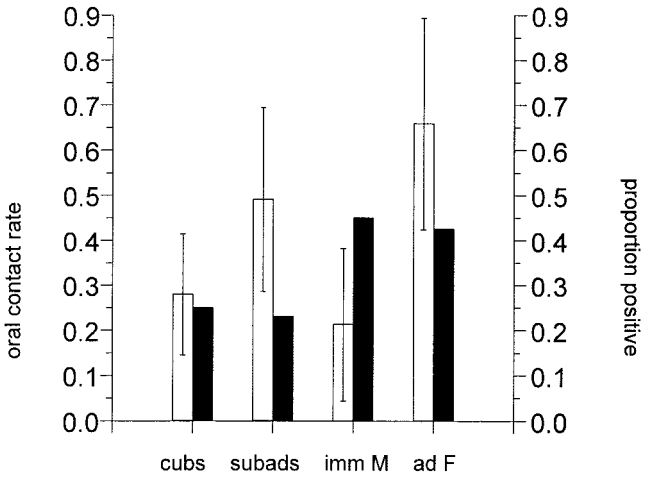

Fig. 1. The mean (±SEM) rate per hour at which individuals had their open mouths licked by other clan members (oral contact rate, open bars), and the proportion of seropositive individuals for each class (solid bars). subads, subadults; imm M, immigrant males; ad F, adult females

Results

Exposure. Rabies VNA titers revealed a high frequency of exposure (37.0%, $n = 100$) to rabies among Serengeti hyenas. Six individuals were sampled twice at intervals between 0.4 and 6.4 years; three initially seropositive animals were seropositive 0.4, 1.2, and 4.7 years later; and three initially seropositive individuals were seronegative 3.9, 4.7, and 6.4 years later. Such changes in VNA titers with time suggest that antibodies persisted in spotted hyenas for many months but not indefinitely. Seroconversion must have occurred sufficiently frequently to maintain seropositivity despite the gradual loss of antibodies through time, e.g., by re-exposure between sampling.

Exposure among males and females was similar, as 37.8% of males ($n = 37$) and 37.3% of females ($n = 59$) had significant VNA titers, and 13.5% of males and 15.3% of females had high (≥1.5 IU/ml) titers. There was no difference in seroprevalence between males and females (VNA titers ≥0.5 IU/ml; $\chi^2 = 0.003$, df = 1, $P = 0.96$; VNA titers ≥1.5 IU/ml: $\chi^2 = 0.06$, df = 1, $P = 0.81$), or between clans ($\chi^2 = 0.15$, df = 2, $P = 0.93$). The probability of having a high (≥1.5 IU/ml) titer, which may be acquired through repeated exposure to the virus or during an active infection, increased with age and social status (logistic regression, log-likelihood ratio test $G = 14.14$, df = 2, $P = 0.00085$, $n = 52$; effect of age: $t = 2.48$, $P = 0.013$; effect of social status: $t = 2.09$, $P = 0.037$). The probability of having merely a positive titer (≥0.5 IU/ml) increased with age ($G = 10.68$, df = 2, $P = 0.0048$, $n = 52$; effect of age: $t = 2.41$, $P = 0.016$) and there was a nonsignificant trend to also increase with social status ($t = 1.72$, $P = 0.085$).

Exposure and Intraspecific Contact. If exposure was a consequence of intraspecific infection via the buccal and nasal mucosa (31), as in some communal species (2, 6), patterns of intraspecific contact rates should follow patterns of seroprevalence in different age and social classes, i.e., increase with age and social status. The mean rate at which individuals had their open mouths licked by other clan members (oral contact rate, Fig. 1) increased with social status (general linear model on $\log_{10}$-transformed rates, $F_{1,73} = 11.22$, $P = 0.0013$) and differed significantly between classes of clan members (den-bound cubs, free-roaming subadults, immigrant males, and adult females, $F_{3,73} = 3.00$, $P = 0.036$), after the total number of potential adult licking partners was taken into account ($F_{1,73} = 0.01$, $P = 0.89$). Cubs and immigrant males had significantly lower oral contact rates than adult females (Fisher's least significant difference test, $P = 0.026$ and $P = 0.01$, respectively, Fig. 1). Also, VNA titers increased with oral contact rates in those 18 individuals for which both titers and contact rates were recorded (Spearman's $\rho = 0.59$, $n = 18$, $P < 0.02$). Thus, the results for oral contact rate confirm this prediction except for adult immigrant males who are more likely to be exposed than expected from oral contact rates (Fig. 1). Rates at which adult males and females received bite wounds from conspecifics (bite contact rate) also increased with social status ($F_{1,277} = 21.89$, $P < 0.00001$) and were similar for both sexes ($F_{1,277} = 1.85$, $P =$ not significant), as expected, and thus conformed to the predicted pattern.

Infection and Lack of Symptoms. Fifty-seven brain samples from Serengeti hyenas killed by motor vehicles or other causes and screened with NCC were negative, but rabies virus was detected by RT-PCR in 3 of 23 hyena brain samples (Table 1). No hyena displayed behavior symptomatic for rabies during our long-term study (or a previous study, ref. 32, in the Serengeti). This lack of symptoms contrasts with the wealth of symptoms observed in cubs of the same study population dying from clinically confirmed canine distemper (33).

Rabies and Hyena Survival. Duration of survival of seropositive hyenas was long, as 50% of such individuals survived for more than 4.4 years after blood sampling, and was unaffected by exposure to rabies (survivorship analysis, incorporating 13 individuals still alive as right-censored (34) data, log-rank test, $\chi^2 = 0.085$, df = 1, $P = 0.77$, $n = 52$, after controlling for the duration of monitoring). There was also no association between longevity and exposure (survivorship analysis, log-rank test, $\chi^2 = 2.90$, $P = 0.09$, $n = 51$). For both survival and longevity there was a nonsignificant trend that, contrary to expectation, seropositive individuals lived longer. High seroprevalence was thus not associated with symptomatic rabies or decreased survival.

Virus Excretion. Rare cases of apparently healthy domestic dogs intermittently excreting virus in their saliva have been reported (3). If such "carriers" exist in wild carnivore populations, they may help maintain rabies in these populations (3, 5). To test this idea, serial saliva samples were obtained from 89 hyenas; we know of no other reports of such sampling. All 303 saliva samples tested negative with NCC, however, RT-PCR detected the presence of rabies virus in 8 of 30 saliva samples from 5 of 11 (45.5%) individuals with positive VNA titers, including two individuals with titers of 0.5 and 0.6 IU/ml. These saliva-rabies-positive individuals survived on average 4.8 years ($n = 5$) after blood sampling and did not display symptomatic rabies.

Basic Reproductive Rate R_0 in Serengeti Hyenas. R_0, the number of susceptible hyenas exposed to the virus by one infectious vector (24), was 1.9 [1.8 or 2.1 if μ is based on (μ + SEM) or (μ − SEM)]. With an estimated population of ≈5,300 hyenas on the Serengeti plains (12), the number of vectors N_v is 470 animals [505 or 420 for (μ + SEM) or (μ − SEM)], or 9% of the population.

Rabies Isolates in Serengeti Carnivores. Rabies virus was isolated from a high proportion of brain samples from Serengeti bat-eared foxes and white-tailed mongooses (*Ichneumia albicauda*) by both NCC and RT-PCR (Table 1). Bat-eared fox carcasses chewed by and retrieved from spotted hyenas were significantly more likely to be rabies-positive than those killed by motor vehicles (NCC: 3 of 4 versus 0 of 9, Fisher exact test, $P = 0.014$; RT-PCR: 4 of 4 versus 1 of 8, Fisher exact test, $P = 0.01$). In

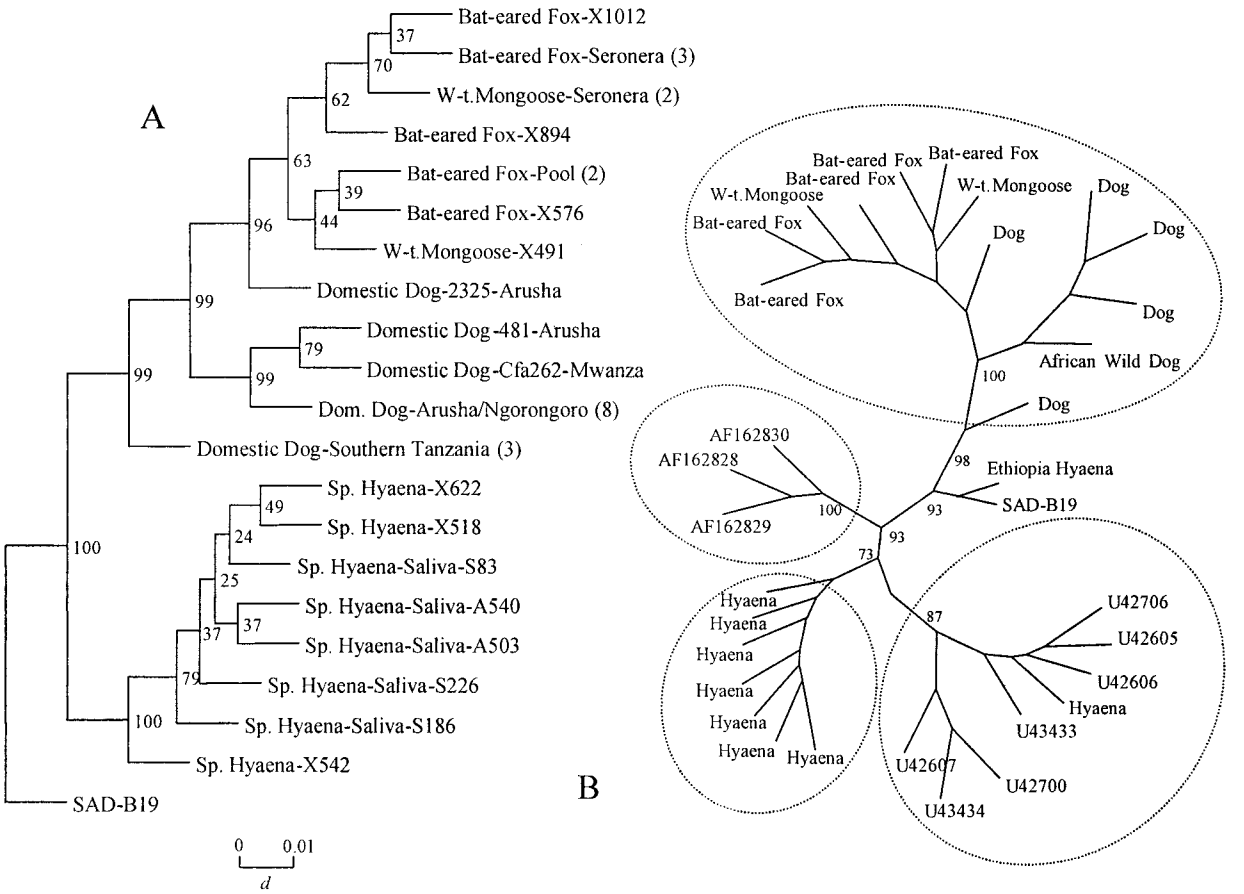

Fig. 2. (*A*) Phylogenetic relationship between rabies viruses isolated from 19 wild carnivores from the Serengeti National Park, 11 domestic dog isolates from within 90 km, and 3 domestic dog isolates from areas in Tanzania ≈390–560 km southeast of the Park. Neighbor-joining tree (*Ln* = −1,047.5) of the maximum-likelihood distances *d* calculated for a 381-bp segment of the N-P gene. The attenuated rabies virus vaccine strain SAD B19 was used as an outgroup. Branch support was obtained from bootstrapping with 1,000 pseudoreplicates. Indicated are the species, location, and identification number of the sample. Numbers in parenthesis indicate number of individuals with identical sequences. (*B*) The rabies virus isolates from Serengeti spotted hyenas are closely related to rabies virus strains of genotype 1 from Europe and the Middle East, and only distantly related to the isolates circulating in Serengeti wild canids and viverrids, local domestic dogs and the African wild dog. The tree (unrooted) was reconstructed as described for *A* by using a 277-bp segment of the nucleoprotein gene from database sequence files representing human and animal examples of rabies virus genotype 1. Indicated are either the source species or the accession number of the database (accession nos. starting with AF from Israel, with U from elsewhere in Europe or the Middle East).

brain-positive bat-eared foxes, both NCC and RT-PCR demonstrated virus presence in salivary glands, and RT-PCR demonstrated virus excretion in saliva of a bat-eared fox with paralytic symptoms (NCC was negative). Partial genetic sequencing of the N-P gene segment of viral isolates from spotted hyenas, canids (African wild dog, bat-eared fox) and viverrids (white-tailed mongoose) from the Serengeti revealed a common canid type. Sequence divergence within canids and viverrids was minor (2.1 ± 0.7%), as was that within the isolates from Serengeti spotted hyenas (1.2 ± 0.5%, Fig. 2*A*). Sequence divergence between the isolates from spotted hyenas and those from canids and viverrids was substantial (8.5 ± 1.2%), as was that between the Serengeti hyenas and the isolate from the African wild dog (9.4 ± 3.1%, Fig. 2*B*). A comparison with sequences of rabies virus from elsewhere showed that the isolates from Serengeti spotted hyenas are closely related to European and Middle Eastern isolates of rabies genotype 1 (Fig. 2*B*), whereas isolates from the Serengeti canids and viverrids occupy a distinct branch closely related to those from Tanzanian domestic dogs (Fig. 2 *A* and *B*). Thus, despite potential contact with bat-eared fox isolates, Serengeti spotted hyenas maintain an apparently less virulent genetic strain of canid type independent of the canid/viverrid community in the same ecosystem.

Discussion

Our results demonstrate that Serengeti hyenas maintain a genetically distinct rabies strain of canid type independently of the canid/viverrid community. The level of exposure to rabies among Serengeti hyenas was higher than in a variety of species in other localities (2, 6, 28, 29). Exposure did not produce life-long persistence of VNA, a phenomenon observed elsewhere (29). Although 37% of Serengeti hyenas were exposed to rabies, infection occurred in only 13% of animals, indicating that many animals eliminated the virus from their body after exposure. As rabies was detected in hyena brains and saliva by RT-PCR, but not by NCC, it is likely that these tissues contained low viral loads. This observation contrasts with the much higher percentage of PCR-positive brain samples in white-tailed mongooses and bat-eared foxes that were also positive by NCC (Table 1). We demonstrated the presence of intraspecific carriers in a free-ranging carnivore with small amounts of virus in their saliva. These carriers were asymptomatic, and their survival and longevity was unaffected by infection.

Rabies Ecology. Although some bat (2, 6) and rodent (7) species are exceptions to the rule that rabies kills the great majority of exposed individuals, no carnivore besides the Serengeti spotted

hyenas have been reported with high seroprevalence that shows asymptomatic infection without loss of survival or longevity. We demonstrate a carrier state in a free-ranging population, and the maintenance of two distinct rabies variants by host species that belong to the same community. Finally, this study demonstrates that social status affects the likelihood of exposure such that high-ranking hyenas are the most likely to be exposed.

Two Distinct Rabies Isolates in the Same Carnivore Community. The rabies isolate found in Serengeti bat-eared foxes and white-tailed mongooses was virulent to these hosts. In both species, outbreaks of rabies were localized and sporadic, and linked in time and space, suggesting the occurrence of cyclic disease episodes (see also ref. 11). In contrast, infection in hyenas by the hyena isolate was asymptomatic and did not reduce survival or longevity. All evidence points to separate processes maintaining both rabies isolates, and to intraspecific maintenance of the Serengeti hyena isolate by social interactions within spotted hyena clans.

Serengeti spotted hyena clans are large and live at high population density (12). Conflicts between clan members may result in fights (13, 14) and in rabies-infected saliva being inoculated into bite wounds. Transmission of rabies virus is also possible when carriers lick the open mouths of other group members (31). Because bite and oral contact rates matched the pattern of seroprevalence in hyenas, the hyena isolate is most likely maintained within the spotted hyena population. Although it cannot be excluded that other species are involved, there is currently no evidence that the hyena isolate circulates within canid or viverrid species.

As the observations of spotted hyenas chewing rabies-positive bat-eared foxes demonstrate, spotted hyenas could be exposed to the bat-eared fox isolate, but there is no evidence that such exposure caused infection. Because of the substantial differences between the Serengeti hyena viral isolates and those of canids and viverrids, it is unlikely that spotted hyenas were the source of rabies virus that killed several African wild dog packs in the Serengeti ecosystem (5, 10).

No distinct viverrid strain of rabies was found in the Serengeti, as described for the yellow mongoose (*Cynictis penicilata*) in Southern Africa (35), and our study is further evidence that canid rabies may infect viverrid hosts (36).

Serengeti Hyena Demography. As with many infectious diseases (24), exposure to rabies among Serengeti hyenas increased with age but did not result in mortality. In contrast, when a virulent strain infected Kalahari spotted hyenas, the resulting age structure in the population was heavily biased against older age classes, suggesting that rabies mortality was chiefly among older individuals and producing an age structure that profoundly differed from that in the Serengeti population (30).

Social Status, Stress, and Exposure. Acute social stress may elevate levels of corticosteroids and depress immunity (37, 38). During periods when coalitions of female hyenas battle for high social status, levels of corticosteroids in Serengeti females are acutely elevated (38). Intense aggression among females during such battles might increase the probability of infection through bites, and elevated stress might compromise immunity and increase the chance of viral infection because of an insufficient immune response to challenge. If high VNA titer levels are produced by asymptomatic rabies infection, then the relationship between social dominance and high VNA titers might indicate an increased probability of infection among high-ranking animals. In that case, high-ranking females are important for rabies transmission within the clan, as they are preferred social partners for other females (39) and preferred courting partners for high-ranking immigrant males (13).

Alternatively, high VNA titers may be the consequence of enhanced seroconversion caused by repeated noninfectious exposure. In that case, high titers might be the result of high oral contact rates, because oral contact rates matched the age-related and social-status-dependent increases in seroprevalence, and are less likely than bite contact to cause infection (28, 31).

Population Structure and Exposure. Our estimate of the basic reproductive rate R_0 of the virus is lower than the rates recorded for most human infectious diseases (24). It may be so low because rabies transmission in Serengeti hyenas does not involve the alteration of the behavior of infected individuals, unlike many other carnivore species (2) and spotted hyenas elsewhere (30).

The simple model used to estimate R_0 assumes a weakly homogenous host population (24). Because exposure was age-specific and linked to social status, the Serengeti hyenas are unlikely to meet this assumption, and thus our estimate of R_0 is a rough rather than precise estimate. An appropriate model for R_0 would have to include age-specific transmission, the effect of social status, groups as distinct units, and the inclusion of a carrier state. However, Serengeti hyenas regularly forage outside their territories in areas that contain large numbers of migratory prey (12, 14). In such areas, fights between individuals from different clans at feeding sites would facilitate viral transmission between clans. Thus, viral transmission in Serengeti hyenas, though primarily based on within-group processes, is likely to be more homogeneous than has been observed in a low-density territorial population in the Kalahari (30).

The Evolution of Low Virulence. Elsewhere in Africa, the rare occurrence of rabid spotted hyenas suggests that challenge can be fatal and typically results in furious rabies (4, 30). Which factors are responsible for variation in virulence between different populations of the same host? Traditionally, it has been assumed that virulent infectious pathogens will evolve to be benign to their hosts, suggesting that, in contrast to other spotted hyena populations, the Serengeti hyena–rabies association is coevolved and has been established for some time. Evolutionary theory and evidence from various host–pathogen systems cast considerable doubt on this assumption, because evolutionary arms races and several other factors can influence virulence (40). Variation in virulence could also be caused by differences in antigenic strains (1, 2, 41), passage of the virus through another host (42, 43), or the mode of infection (2, 28, 31).

As the evidence points to intraspecific transmission, passage of the virus through another host is unlikely. Because the Serengeti hyena isolate substantially differs from a virulent strain (17) in an Ethiopian spotted hyena (Fig. 2*B*), antigenic differences may affect virulence. The virulence of the Serengeti hyena strain may also be limited by a high degree of immunity achieved through frequent exposure to small viral loads during social contact. Rabies viral load in infected hyena organs and the saliva of carriers was low, and the transfer of small viral loads via the buccal–nasal route may boost the immune response if it leads to enhanced seroconversion. Because many individuals are likely to be already protected through high seroprevalence, occasional intraspecific infection from bite wounds might cause asymptomatic rabies infection in only a small proportion of hyenas.

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